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EXOPOLYSACCHARIDE-PRODUCING LACTIC ACID BACTERIA – HEALTH-PROMOTING PROPERTIES AND APPLICATION IN THE DAIRY INDUSTRY

Anna M. Berthold-Pluta[1], Antoni St. Pluta[1], Monika Garbowska[1], Lidia Stasiak-Różańska[2]

[1] Division of Milk Biotechnology, Department of Biotechnology, Microbiology and Food Evaluation, Faculty of Food Sciences, Warsaw University of Life Sciences – SGGW
[2] Division of Biotechnology and Microbiology, Department of Biotechnology, Microbiology and Food Evaluation, Faculty of Food Sciences, Warsaw University of Life Sciences – SGGW

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Abstract: Exopolysaccharides (EPS) are one of the classes of extracellular biopolymers synthesized by bacteria. Some strains of lactic acid bacteria (LAB) used in the dairy industry are able to synthesize EPS (EPS(+) strains). EPS may be secreted by a cell in the form of capsule or slime. Our review describes the factors influencing the activity of EPS production by LAB, the impact of the use of EPS(+) strains on the quality of fermented milk products (yoghurt, cheeses, etc.) and pro-health properties of EPS produced by LAB. The capability to synthesize EPS by LAB depends on many factors, e.g., affiliation to species and characteristics of strain, growth stage, composition of culture medium (type of carbon and nitrogen sources, and presence of other nutrients), temperature, pH, and presence of adjuvant microflora. The presence of EPS synthesized by LAB strains has a significant effect on changes in various properties of dairy products, including: yoghurt, kefir and many other fermented milk drinks, sour cream and cheeses. The EPS act as thickening, emulsifying and gelling agents, hence the use of EPS(+) strains may become a certain alternative to the use of thickeners in, e.g., fermented milks. During formation of a casein milk curd, EPS are able to bind water and thus reduce syneresis. The high water holding capacity of EPS has a positive effect on increasing viscosity and improving texture of low-fat cheeses. EPS are claimed to have health-promoting properties, like: anticarcinogenic, antioxidative, immunomodulatory and reducing blood cholesterol.

BAKTERIE MLEKOWE WYTWARZAJĄCE EGZOPOLISACHARYDY – WŁAŚCIWOŚCI PROZDROWOTNE I ZASTOSOWANIE W PRZEMYŚLE MLECZARSKIM

Streszczenie: Egzopolisacharydy (EPS) to jedna z klas biopolimerów pozakomórkowych wytwarzanych przez bakterie. Niektóre szczepy bakterii kwasu mlekowego (LAB) stosowane w przemyśle mleczarskim są w stanie syntetyzować EPS (szczepy EPS (+)). EPS mogą być wydzielane przez komórkę w postaci kapsułek lub śluzu. W niniejszym przeglądzie opisano czynniki wpływające na aktywność wytwarzania EPS przez LAB, wpływ stosowania szczepów EPS(+) na jakość fermentowanych produktów mlecznych (jogurty, sery, itp.) oraz prozdrowotne właściwości EPS wytwarzanych przez LAB. Zdolność wytwarzania EPS przez LAB zależy od wielu czynników, np. przynależności do gatunku i charakterystyki szczepu, fazy wzrostu, składu pożywki hodowlanej (rodzaj źródła węgla i azotu oraz obecność innych składników odżywczych), temperatury, pH i obecności mikroflory towarzyszącej. Obecność EPS wytwarzanych przez szczepy LAB ma istotny wpływ na zmiany różnych właściwości produktów mlecznych, w tym: jogurtów, kefirów i wielu innych mlek fermentowanych, śmietany oraz serów. EPS zachowują się jak środki zagęszczające, emulgujące i żelujące, a zatem użycie szczepów EPS(+) może stać się pewną alternatywą dla zastosowania środków zagęszczających, np. w mlekach fermentowanych. Podczas tworzenia się skrzepu kazeinowego, EPS mogą „wiązać" wodę i tym samym zmniejszać synerezę. Wysoka zdolność zatrzymywania wody przez EPS ma pozytywny wpływ na zwiększenie lepkości i poprawę tekstury serów, zwłaszcza tych o obniżonej zawartości tłuszczu. Wiadomo także, że EPS mają właściwości prozdrowotne, takie jak: przeciwnowotworowe, przeciwutleniające, immunomodulujące i obniżające poziom cholesterolu we krwi.

Key words: EPS, LAB, fermented milk products, dairy products
Słowa kluczowe: EPS, bakterie mlekowe, mleczne produkty fermentowane, produkty mleczne

* Corresponding author: dr. Anna Berthold-Pluta, Division of Milk Biotechnology, Department of Biotechnology, Microbiology and Food Evaluation, Faculty of Food Sciences, Warsaw University of Life Sciences – SGGW, Nowoursynowska 159C St, 02-787 Warsaw, Poland; tel. +48 22 59 37 668; e-mail: anna_berthold@sggw.pl

1. Introduction

Apart from their basic metabolic activities, bacteria are also capable of synthesizing many biopolymers which differ in structure and chemical properties and thus in their functions in the cells. Considering their localization, biopolymers may be divided into intracellular (a small group with limited applications) and extracellular (a large group with wide applicability) ones. One of the classes of extracellular biopolymers includes exopolysaccharides (EPS). The EPS may be secreted outside a bacterial cell or may be produced as a capsule bound with external cellular membranes [66]. The EPS serve various functions in bacterial cells, like: protecting them against adverse effects of the environment (e.g., high or low temperature, high or low pH, and toxic metal ions) and against biological factors (e.g., phage attack), or helping them to colonize the environment (they are constituents of biofilms). It is presumed that EPS do not serve as a source of energy to bacterial cells, though some probiotic strains of lactic acid bacteria (LAB) were shown to be capable of EPS degradation [75]. Some LAB strains used in the dairy industry to produce fermented milks, e.g., yoghurt, kefir, sour milk and other fermented milk drinks, are able to synthesize EPS (the so-called EPS(+) strains). The application of EPS(+) strains may have highly positive effects on the rheological properties and quality of the manufactured fermented products [7, 12]. The EPS produced by lactic acid bacteria during formation of the casein curd of milk are capable of water retention and thereby inhibit syneresis in fermented drinks. In addition, by reacting with proteins, they may contribute to the reinforcement of the casein network, which improves the rheological properties, quality of the final product, and cheese yield. The use of adjunct EPS(+) starter cultures improves the smoothness, viscosity and stability of a yoghurt gel and of other fermented milks [4, 22, 32]. The character of changes induced in fermented products by the presence of EPS is determined by the chemical composition and structure of these compounds, including e.g., their molecular weight, type of bonds and the presence of side chains. The rheological properties of EPS-containing food products are also affected by the time of their most active production by LAB during food manufacture [21, 22, 67, 75]. The most active LAB strains were shown to produce EPS at even 3 g/l [58]. The EPS display also some health-promoting properties. An increase in the viscosity of EPS-containing products is believed to extend the time of their gastrointestinal passage, which may be beneficial for temporary gut colonization by LAB [15]. In addition, many studies have shown the immunomodulatory, hypocholesterolemic, anti-carcinogenic, and anti-ulcerous activities of EPS [24, 26, 42, 43, 50, 56, 59].

2. General characteristics of exopolysaccharides

The EPS are high-molecular, long-chain linear biopolymers with side chains, which are constituted by carbohydrate units linked with α- and β-glycosidic bonds. They may be secreted by a cell to the extracellular space and remain bound with its surface thus forming a capsule (CPS – capsular exopolysaccharides). EPS may also be released to the external environment in the form of slime exopolysaccharides. Other types of polysaccharides include, e.g., cell wall polysaccharides (CWPS) linked with ionic or covalent bonds with the peptidoglycan layer on the cell's surface [88]. Taking into account the EPS structure, they may be divided into homopolysaccharides (HoPS) and heteropolysaccharides (HePS). Molecules of HoPS consist of successively repeated monosaccharides of one type (e.g., D-glucose or D-fructose), and include two major groups: glucans (dextran, mutan, alternan, reuteran, curdlan) and fructans (levan, inulin-type fructans) [63, 67, 88]. In turn, HePS are built of sub-units containing 3 to 8 monosaccharides: D-glucose, D-galactose, L-fructose, L-rhamnose or, alternatively, acids: D-glucuronic, L-glucuronic and D-mannuronic. The HePS may also contain amino sugars, like, e.g., N-acetyl-D-glucosamine or N-acetyl-D-galactosamine [7, 67]. Molecular weights of HePS range from 10^4 to 6×10^6 Da [7].

Glucans – being representatives of HoPS – are divided into α-D-glucans and β-D-glucans [63]. The production of α-D-glucans (e.g., dextran, mutan, alternan, reuteran) is assisted by dextransucrase which is an extracellular enzyme synthesized by, among others, bacteria of the *Leuconostoc*, *Streptococcus*, and *Lactobacillus* genera. In turn, β-D-glucans (e.g., curdlan) contain glucose residues linked with β-1,3-glycosidic bonds. The LAB capable of their production include strains from *Pediococcus*, *Oenococcus*, and *Lactobacillus* genera [7]. One of the HoPS α-D-glucans is dextran. The dextran molecule synthesized by *Leuconostoc mesenteroides* has a linear structure and is built mainly of D-glucose residues (95%) linked with α-1,6-glycosidic bonds. The remaining part is constituted by side α-1,3-glycosidic bonds. Differences in the structure of dextrans isolated from various LAB include mainly: type, number and arrangement of side chains in a molecule. Bacterial strains which produce dextran include strains from *Leuconostoc*, *Streptococcus* and *Lactobacillus* genera. In the pure form, dextran is applied as a component of gel used for filtration (Sephadex) and as a blood substitute [7, 36]. Another example of α-D-glucan is mutan, which is built of D-glucose molecules linked in over 50% with α-1,3-glycosidic bonds. A high activity of the mutan-producing enzyme was reported in *Ln. mesenteroides* NRRL B-523 and B-1149 strains and in some strains from the genus *Streptococcus*. Mutan is respon-

sible for the adhesion of oral cavity microflora to teeth surface, which contributes to the formation of dental plaque and calculus [7, 63]. Alternan – which contains alternatively arranged α-1,6 and α-1,3-glycosidic bonds – is produced by an enzyme called alternansucrase. Capability for alternansucrase synthesis has so far been reported for three strains of *Ln. mesenteroides*: NRRL B-1355, NRRL B-1501, and NRRL B-1498 [63]. In turn, reuteran is an α-D-glucan produced by reuteransucrase enzyme isolated from *Lactobacillus reuteri* 121 and ATCC 55730 [44, 45]. Curdlan is a neutral, gel-forming β-D-glucan with a straight chain [48]. Curdlan and other polysaccharides belonging to this group were described as anti-carcinogens activating macrophages and leukocytes [7].

One of the most extensively described HePS is kefiran, built of mannose, glucose and galactose in approx. ratio of 1:5:7 [87]. Ability to produce kefiran was reported for: *Lactobacillus kefiranofaciens*, *Lb. kefirgranum*, *Lb. parakefiri*, *Lb. kefir*, *Lb. plantarum*, and *Lb. delbrüeckii* subsp. *bulgaricus* [3, 84].

3. Factors affecting exopolysaccharides synthesis by lactic acid bacteria

The capability to synthesize EPS by LAB varies and depends on many factors, and is species- and strain-specific. EPS production by lactic bacilli (*Lb. plantarum*) accounts for ca. 0.14 g/l [81], *Lb. bulgaricus* – 0.06–0.15 g/l [83] and *Lb. fermentum* – 0.75 g/l [41]. In turn, lactic streptococci (*S. thermophilus*) are able to produce 0.1 g/l [89]. However, the greatest production of EPS was demonstrated for *Lb. rhamnosus* RW-9595M strain (2.8 g/l), *Lb. kefiranofaciens* WT-2B strain (2.5 g/l) and *Lb. plantarum* BR2 strain (2.8 g/l) [58, 60, 79].

EPS production is determined by the growth stage of bacteria, composition of culture medium (type of carbon and nitrogen sources, and presence of other nutrients), temperature, and pH, and/or by the presence of adjuvant microflora [2, 3, 62, 72, 82, 86, 92, 93] (Tab. I). The concentration of produced EPS is largely affected by conditions of growth of bacteria which synthesize them, whereas the monosaccharide composition of most of the EPS does not depend on the available source of carbon. Interesting – especially from the perspective of practical application – seems to be the fact that the same LAB strain may produce different EPS under various growth conditions [39].

Ample studies have addressed the effect of culture medium composition on the concentration of EPS produced by LAB [69, 72, 82, 86, 92]. The *Lb. casei* and *Lb. bulgaricus* strains were shown to produce EPS in the concentration below 0.6 g/l when cultured in fermented milk, and at 1.5 g/l when grown in M17 broth

Table I
Factors affecting EPS synthesis by lactic acid bacteria

Factors affecting EPS synthesis by LAB	References
Species/strain	[41, 58, 60, 79, 81, 83, 89]
Growth stage of bacteria	[12, 13, 93]
Temperature	[12, 13, 49, 62, 72, 82, 92, 93]
pH of medium	[12, 39, 82, 93]
Time of incubation	[12–14, 53, 82, 93]
Culture medium composition (e.g., source of nitrogen and carbon)	[69, 72, 82, 83, 86, 92, 93]
Presence of adjuvant microflora	[2, 3]

enriched with various sources of carbon and nitrogen [69]. In turn, Rabha *et al.* [72] demonstrated milk to be a better medium for EPS production by *S. thermophilus* than MRS or M17 broths. Investigations on the effect of carbon source on EPS concentration have demonstrated that increased synthesis of kefiran by LAB engaged in kefir production was achieved with disaccharides used as sources of carbon. The enrichment of culture medium in saccharose or lactose enabled achieving kefiran content in kefir grains at 3.8% and 4.3%, respectively. When fructose or glucose were used in the culture medium as the source of carbon, the respective values were lower and reached 2.7% and 2.1% [92]. *Lb. fermentum* F6 produced greater amounts of EPS when glucose was used as a carbon source in the culture medium (ca. 0.035 g/l), compared to fructose > lactose > galactose [93]. *S. thermophilus* strain 23, isolated from homemade yoghurt in Bulgaria produced more EPS in the presence of sucrose (0.13 g/l) than in the presence of lactose (0.083 g/l) [83]. Results of this research show milk to be a good culture medium for EPS production by LAB, especially when the milk composition is modified by addition of a source of carbon. EPS production by LAB is also affected by the source of nitrogen in the culture medium. The highest concentration of kefiran was produced in the presence of organic nitrogen, e.g., casein (1.78 g kefiran per l), peptone (1.65 g/l), tryptone (1.64 g/l), or yeast extract (1.64 g/l), whereas a significantly lower amounts were noted when the culture medium was supplemented with inorganic nitrogen (urea – 0.89 g kefiran per l, ammonium chloride – 0.73 g/l, ammonium sulfate – 0.69 g/l) [86]. Similar results were reported by Zajšek *et al.* [92]. In their study, the content of kefiran in kefir grains was significantly lower when ammonium chloride was used as the source of nitrogen in the culture medium (1.3%), whereas its content increased to 1.8% when skim milk was used as the source of organic nitrogen (casein) [92]. Additional enrichment of the skim milk-based culture medium with peptone and yeast extract improved EPS

production also by *S. thermophilus* (2 to several times more, depending on the strain) [82].

Another factor influencing EPS synthesis by LAB is temperature. Many studies have shown the highest production of EPS at the so-called sub-optimal temperature, i.e. at few °C lower than the optimal temperature for growth of a given LAB species, by both mesophilic and thermophilic species [62, 72]. The overproduction of EPS at the sub-optimal temperature is a likely response of a bacterial cell to the physiological stress induced by the decreased temperature, especially in species or strains defective in proteolytic activity (e.g., *S. thermophilus*) [12, 72, 82]. In their research on thermophilic *S. thermophilus* BN1 with the optimal growth temperature at 42°C, Rabha *et al.* [72] demonstrated a significantly higher EPS production by this strain at a slightly lower than optimal growth temperature (37°C), irrespective of the culture medium composition. For example, in skim milk, EPS production by this strain reached 0.097 g PDM (polymer dry mass) per l at 42°C, but was by over 5-fold higher at 37°C. In their study on kefiran synthesis, Zajšek *et al.* [92] demonstrated the highest content of EPS in kefir grains at the temperature of 25°C (2.75%), and the lowest one – at 37°C (1.3%). In turn, the latter temperature turned out to be optimal for the growth of the kefir grains. In addition, the temperature of fermentation was found to influence the galactose to glucose ratio in the produced kefiran. At the temperature of 25°C, this ratio was lower (ca. 1.3) than at the temperature not facilitating EPS production (ca. 1.4). The temperature lower than the optimal growth temperature was also reported to enhance EPS production by *Lb. sake* 0–1 [13]. In turn, Zhang *et al.* [93] demonstrated that *Lb. fermentum* F6 produced the highest concentration of EPS when cultured at the optimal temperature (37°C). Likewise, strains of *Lb. fermentum* TDS030603 and *Lb. casei* CRL 870 were shown to produce the highest concentrations of EPS at the optimal temperature for their growth, i.e. 37°C [49]. Also Mende *et al.* [62] demonstrated higher EPS production by the *Lb. delbrüeckii* subsp. *bulgaricus* DSM 20081 strain at temperatures from 30°C to 40°C (optimal growth temperature for this strain is 40°C), while almost by half lower EPS production at 45°C. Thus, it may be concluded that, unlike the sub-optimal temperature, the temperature exceeding the optimal growth temperature does not enhance EPS synthesis as a form of cell response to physiological stress. Ruas-Madiedo *et al.* [76] showed no effect of temperature on EPS concentration in milk fermented by *Lc. lactis* subsp. *cremoris* strains. In turn, they observed significant differences in EPS concentrations produced in replications of the same experiment. This may result from the fact that the capability to produce EPS is not a permanent trait, especially when strains are subjected to multiple proliferation due to the applied experimental method, which may lead to the loss of genes responsible for EPS synthesis that are located on plasmids. In addition, prolonged incubation may induce hydrolytic degradation of EPS.

Another factor influencing LAB ability to produce EPS is the time of incubation. Zhang *et al.* [93] showed the growth stage of *Lb. fermentum* F6 to be associated with EPS synthesis, the concentration of which decreased at the end of fermentation in the stationary phase of growth (after 32 h). Presumably, the *Lb. fermentum* F6 strain produces glycohydrolase which catalyzes degradation of polysaccharides, thereby decreasing EPS synthesis. EPS degradation after prolonged incubation was also observed in cultures of other LAB strains [12, 13]. Decreased EPS degradation was demonstrated when bacteria were incubated at a lower temperature and pH than the ones optimal for their growth [14, 53]. In the case of some LAB (e.g., *S. thermophilus* ST111), the concentration of EPS did not change over time, as the maximum yield of their synthesis occurred at the end of fermentation [82].

The optimal pH value for EPS production varies between species and between LAB strains, however usually reaches around 6 [12]. The intensive growth and maximum capability to produce EPS by *S. thermophilus* ST111 were determined in the culture medium with active acidity of 6.2 [82]. In turn, the optimal pH for EPS synthesis by *Lb. delbrüeckii* subsp. *bulgaricus* is similar to the optimal pH for their growth (ca. 6.5) [39]. Finally, Zhang *et al.* [93] demonstrated the highest EPS production by the *Lb. fermentum* F6 strain at pH 6.5, and a higher cell count of these bacteria at a slightly higher pH value (ca. 7.0).

One of the factors which influence EPS synthesis by some LAB strains is the simultaneous presence of other LAB in the culture medium. This issue is of high importance as mixed cultures constituted by several strains of the same LAB species or by different species are usually used in the industrial practice. Mechanisms of their interactions may be based on cooperation (e.g., *Lb. bulgaricus* and *S. thermophilus* in yoghurt), but also on competition or even growth inhibition (production of bacteriocins). Investigations on the effect of the mixed culture of the EPS(+) strain *Lb. kefiranofaciens* ZW3 with the EPS(–) strains: *Lb. bulgaricus* and *S. thermophilus*, demonstrated that the ZW3 strain produced EPS of a different structure compared to the EPS from a culture without yoghurt bacteria [3]. The mixed culture of EPS(+) and EPS(–) strains may, therefore, offer the possibility of changing the structure and type of the produced EPS and – indirectly – of inducing highly specified, desired changes in the final product.

4. Effect of exopolysaccharides on the quality of fermented milk products

The capability of LAB to produce EPS in milk during fermentation is an especially important trait for the dairy industry as these compounds increase the apparent viscosity and improve the texture and mouthfeel of the dairy products as well as inhibit syneresis even at their low concentrations (from 0.1 to 0.4 g/l) [17]. The presence of EPS synthesized by LAB strains has a significant effect on changes in various properties of dairy products, including: yoghurt, kefir and many other fermented milk drinks, sour cream and cheeses [15, 28, 32, 46].

The consumption of milk desserts, yoghurts and snacks is observed to successively increase in the United States and also in EU Member States. Products of this type contain additives which affect their rheological properties; but, on the other hand, they have to meet consumers demands for natural and healthy foods [54]. In Great Britain, the addition of stabilizers is regulated by law, e.g., the addition of starch and other stabilizers to yoghurts should not exceed 1% and 0.5%, respectively. It seems that in this respect the EPS(+) strains of LAB may arise interest of the dairy industry. The use of EPS(+) starters strongly inscribes itself into the "clean label" trend which is rather a permanent and irreversible trend that needs to be taken into account by food producers [57].

The EPS(+) LAB strains are applied as adjunct starters in the manufacture of fermented products or are incorporated into mixed starters (Tab. II). The effect of EPS on the rheological properties of fermented milk is more tangible and yields better outcomes when EPS are synthesized *in situ* in the product than when they are added as one of the components [15, 46]. Such an approach responds to economic concerns but may be applied mainly in the case of fermented products.

Viscosity of milk gels formed during fermentation by the EPS(+) LAB strains depends not only on the quantity of EPS products but also, to a significant extent, on their primary structure (stiffness of the EPS

Table II
The use of EPS(+) lactic bacteria in the production of various fermented milk products

LAB species	Products
Lactococcus	
Lc. lactis subsp. *lactis*	buttermilk, kefir, Nordic ropy milks
Lc. lactis subsp. *cremoris*	buttermilk, kefir, dahi, Nordic ropy milks, reduced-fat Cheddar cheeses
Lc. lactis subsp. *lactis* biovar diacetylactis	buttermilk, kefir, dahi, Nordic ropy milks
Streptococcus	
S. salivarius subsp. *thermophilus*	yoghurt, dahi, Nordic ropy milks, fresh cheeses, Mozzarella cheese, Feta cheese
Leuconostoc	
Ln. mesenteroides subsp. *mesenteroides*	kefir, sour cream
Ln. mesenteroides subsp. *cremoris*	kefir, sour cream, Nordic ropy milks
Ln. mesenteroides subsp. *dextranicum*	kefir, sour cream, Nordic ropy milks
Lactobacillus	
Lb. delbrüeckii subsp. *delbrüeckii*	fermented milks, yoghurt
Lb. delbrüeckii subsp. *lactis*	fermented milks
Lb. delbrüeckii subsp. *bulgaricus*	yoghurt, Bulgarian buttermilk, Nordic ropy milks
Lb. helveticus	kefir, kumys, Nordic ropy milks
Lb. acidophilus	acidophilus milk, kefir
Lb. paracasei subsp. *paracasei*	fermented milks
Lb. johnsonii	probiotic yoghurt, fermented milks
Lb. casei	probiotic yoghurt
Lb. paracasei	probiotic yoghurt
Lb. reuteri	probiotic yoghurt
Lb. rhamnosus	kefir, acid-rennet cheeses
Lb. plantarum	kefir
Lb. kefir	kefir
Lb. kefiranofasciens	kefir
Lb. brevis	kefir
Lb. fermentum	kefir

backbone), molecular weight, molecule rotation and charge [21, 22, 74, 75], character of bonds inside the molecule, and potential presence of side chains [21, 22, 46, 47]. At the same molecular weight, EPS molecules with a linear structure occupy a larger volume in solution compared to the branched EPS, owing to which they have a greater impact on increasing the viscosity of a solution. Also the stiffness of the EPS backbone contributes to the increase in the viscosity of the EPS-containing solutions by preventing potential deformations of the molecules. In milk gels, the presence of EPS with high molecular weight, stiff and only slightly branched has a positive impact on their viscosity and stability, and on their reduced syneresis [21]. The high degree of branching and the flexibility of the backbone leads to the "compactness" of the EPS, which results in a decreased viscosity of the solution. The microstructure of a milk gel formed with LAB strains producing EPS of this type is similar to the microstructure of a gel made with EPS(–) cultures [22].

Another factor affecting the rheological properties of milk gels produced with ESP(+) LAB strains is the charge of the EPS molecule. The use of strains synthesizing anionic EPS in starter cultures enabled achieving milk gels with higher values of the elastic modulus (*G'*) compared to the starters containing LAB strains synthesizing neutral EPS. The improvement of milk gels elasticity is probably due to electrostatic interactions between the anionic EPS and the positively charged molecules of casein, which strengthen the casein network [22]. Apart from dynamic changes in casein micelles, colloidal calcium phosphate, whey proteins and other milk constituents which cause the formation of a protein network, the process of milk fermentation by EPS(+) bacteria involves also EPS synthesis and incorporation into the protein network. Due to the successive release of EPS by LAB, increased acidity and change in environment conditions, interactions between EPS and milk proteins are also likely. The equilibrium between repulsion/attraction of EPS and milk proteins varies throughout fermentation and depends mainly on the environment pH as the released casein micelles bear a negative charge in milk with pH > 4.6–6.65, a neutral charge in milk with pH 4.6, and a positive charge in milk with pH below 4.6, whilst the process of fermentation may terminate at different pH values. Understanding the dynamics of this phenomenon allows modifying and controlling final properties of milk gels [23, 46].

The EPS may influence the formation of a casein gel structure by acting as a "bond", and their effect on the protein matrix and structure depends on their concentration, interactions with proteins and characteristics of a molecule [22, 70]. They may positively co-act with milk proteins, thus increasing values of the viscoelastic moduli and firmness of milk gels [70]. This may be caused by electrostatic interactions between casein and EPS, which besides protein-protein interactions may additionally reinforce the structure of a casein gel [6, 21, 23, 70]. Interactions between milk proteins and EPS in a complicated system, like, e.g., fermented milk, are poorly recognized. Research addressing this problem need to take into account the differences in the mechanisms of formation of these compounds during fermentation compared to the process aided with stabilizers (modified starches and pectins) added to milk prior to its souring. Some studies related to the protein-EPS interactions were conducted in model systems, wherein purified EPS preparations were added to milk before fermentation [23]. However, milks produced in this way had different, less beneficial rheological properties than the fermented milks in which EPS were synthesized by LAB *in situ* during fermentation [15]. This may result from the loss of certain properties of EPS (for example ropy character) during purification and drying of their preparations [23]. Results of investigations on the interactions between proteins and EPS in skim milk media do not fully reflect the complexity of milk composition which, apart from casein and whey proteins, includes also fat, lactose and mineral salts. Ayala-Hernández *et al.* [5, 6] in their studies on interactions between whey proteins and EPS with a well-known structure produced *in situ* by *Lc. lactis* in a simplified model of milk (milk permeate) demonstrated interactions between anionic EPS and whey proteins occurring in the amount of 2–8%, at pH 4.5 after 12 h of fermentation at a temperature of 30°C. The interactions between EPS and proteins (both casein and whey proteins) during fermentation of a dairy model system were also confirmed by Gentés *et al.* [22].

A largely significant aspect from the practical perspective is the capability of milk gels to "recover" after stirring and pumping during production of e.g., stirred yoghurts. Studies addressing the effect of stirring milk gels produced using EPS(+) strains and for comparison using EPS(–) strains demonstrated that although the EPS-containing gels were indeed more compact, a greater decrease was noted in the value of their elastic modulus (*G'*) after stirring than in the gels free of EPS [31, 32, 46]. The non-stirred gel formed by the EPS(+) strain showed a network of thick, continuous aggregates of milk with large void spaces, whereas the gel formed by the EPS(–) strain showed a network of fine protein strands and small void spaces around. Provided that a considerable amount of EPS is produced after the onset of aggregation of casein micelles, it would be entrapped in pockets between casein clusters and constrained around the bacteria cells. Such spatial constrain would reduce the probability of possible interactions of EPS with casein micelles and induce an inten-

sification of the mutual interactions of casein particles. Stirring changes the structure of the EPS-containing gel, causing the formation of a larger number of pores and channels and of less compact protein aggregates. In addition, it causes the formation of channels with EPS-containing serum concentrated in larger strands than those observed in the undisturbed gels. This was attributable to the intense mutual interactions and aggregation of EPS molecules in the continuous serum phase due to repulsion with casein micelles. After stirring, the microstructure of the gel formed by the EPS(+) strain showed a significantly lower connectivity between protein aggregates, most likely as a result of the void spaces filled with EPS which surrounded casein. In the EPS(–) gels, stirring resulted in a more aggregated protein network with more connectivity between protein strands and smaller pores compared to the stirred gels containing EPS. The structure of the stirred EPS(–) gel did not differ significantly from its structure before stirring, but was more dense and aggregated [31, 32, 46]. Kristo *et al.* [46] concluded that the time needed to reach the gelation point (T_{gel}) in the case of milk fermented by the EPS(+) *Lc. lactis* subsp. *cremoris* JFR1 strain and time needed to reach pH 4.6 ($T_{pH4.6}$) were longer than for the milk fermented by the EPS(–) strain. However, the presence of EPS caused no significant difference in gelation pH (pH_{gel}).

4.1. Effect of EPS on the quality of yoghurts

Yoghurt is a type of fermented milk manufactured using starter cultures: *Lb. delbrüeckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus*, usually used in the 1:1 ratio. The production of EPS by these strains ranges from 0.057 to 0.424 g/l for *Lb. bulgaricus* and from 0.030 to 0.890 g/l for *S. thermophilus* [7]. The growth of yoghurt bacteria during yoghurt manufacture and their impact on the improvement of textural properties and decrease of syneresis are described as synergistic. Lactobacilli of the *Lb. bulgaricus* species grow first and produce metabolites (mainly amino acids) needed for the growth of *S. thermophilus*, which in turn produces formic acid and CO_2, thereby stimulating the growth of *Lb. bulgaricus.* Bacilli of *Lb. bulgaricus* are mainly responsible for souring, whereas *S. thermophilus* – for the formation of a typical yoghurt flavor [7]. Maintaining the desired consistency of yoghurt is one of the major technological problems in the yoghurt production process. The two most often produced types of yoghurts include set yoghurt (intact curd) and stirred yoghurt (disrupted curd). They differ significantly in their rheological properties: the set yoghurt exhibits traits of a gel, while the stirred yoghurt is a non-Newtonian liquid and a watery, slightly elastic fluid. Unlike in the set yoghurt, damage of the curd structure in the stirred yoghurt may occur at all stages of the production process, i.e. since the moment of curd formation (pH 4.6–4.7) till product packaging [80].

The final consistency of the natural yoghurt is a result of the effects of the milk protein complex, lactic acid, and potentially EPS produced by yoghurt cultures. Desired rheological properties of yoghurt include: hardness, firmness, cohesiveness, smoothness, viscosity, and stability, which when taken all together signify lack of susceptibility to syneresis. The set yoghurt with a high level of syneresis is usually perceived by consumers as having a defect, although this is a natural phenomenon in this product [4, 34]. In the industrial practice, syneresis is reduced through increasing contents of dry matter components in processing milk to 14% (w/w) with dry dairy ingredients (skim milk powder, whey protein isolate, whey protein concentrate, sodium- or calcium caseinates) or by using stabilizers [80]. Unfortunately, the use of these additives always increases production costs of yoghurts, and the addition of stabilizers (gelatin, modified starches, gums) may negatively affect yogurt perception by potential consumers. Some countries have imposed bans or reductions in stabilizers use in yoghurt production. Yoghurts manufactured with strains capable of EPS production are less susceptible to syneresis, have higher viscosity and water holding capacity as well as smoother and creamy texture and decreased granularity. It may be concluded that the use of EPS(+) LAB strains in the production of yoghurts offers the possibility of limiting or eliminating the necessity of applying texture-forming additives [4, 27, 32–34, 70].

The microstructure of yoghurt is built of a casein matrix with incorporated fat globules. Spaces in the gel are filled with serum and LAB cells. The cultures applied in yoghurt production synthesize both capsular EPS and EPS secreted in the form of slime outside the cell. The capsular EPS are in direct contact with cells which use them for incorporation into the protein matrix. Different EPS(+) strains have various effects on the rheological properties of yoghurts. The stirred yoghurts produced using starter cultures synthesizing slime EPS were shown to be more viscous than these produced using cultures synthesizing capsular EPS or using EPS(–) starter cultures [4]. The yoghurt cultures may be divided into three groups: cultures incapable of EPS production, cultures producing capsular EPS, and cultures capable of producing both capsular and slime EPS [16, 34]. The cultures synthesizing slime EPS are generally believed to positively affect yoghurt consistency; however, the overproduction of these EPS leads to the manufacture of products with undesired mucosity, clearly perceptible in the mouth [19, 80]. The capsular EPS are usually not produced in excessive amounts as the size of the capsule is limited by the size of the

bacterial cell. Bacterial capsules loosen gel microstructure in yoghurt, thus making its consistency smoother. Starter cultures synthesizing capsular EPS and lacking slime formation, produce yoghurts which are more viscous, more stable and less susceptible to syneresis compared to yoghurts produced by cultures incapable of synthesizing capsular EPS. In addition, the capsules retard diffusion of lactic acid from the cells, thus lead with time to the arrest of acid production by the cells. This may prevent the over-souring of yoghurt [31, 34]. A comparison of the microstructure of milk fermented using EPS(–) cultures (*S. thermophilus* CHCC 5843 and *Lb. delbrüeckii* subsp. *bulgaricus* CHCC 769), slime cultures (*S. thermophilus* CHCC 3534 and *Lb. delbrüeckii* subsp. *bulgaricus* CHCC 769), highly viscous cultures (*Lc. lactis* subsp. *cremoris* JFR1), revealed a compact protein network being formed in the milk fermented with the EPS(–) cultures, whilst a curd with an open, porous structure – in the milk fermented with EPS(+) cultures. The milk fermented using the slime strain *S. thermophilus* CHCC 3534 developed a structure with greater pores than that fermented with *Lc. lactis* subsp. *cremoris* JFR1 strain producing capsular EPS. In both cases, the EPS were separated from the protein network. In addition, more abrupt syneresis was observed in the milk fermented with EPS(–) than with EPS(+) cultures [34].

A study aimed at elucidating the role of EPS in modeling the structure of yoghurt was conducted with LAB strains capable and incapable of EPS production. Viscosity was always higher in yoghurts manufactured with EPS(+) strains. Importantly, the EPS do not impart their own taste nor aroma to the product, but only improve its texture. The use of LAB cultures producing slime EPS was shown to enable reduction of soy protein isolate or concentrate added during the production of stirred yoghurt [4]. Partial or complete substitution of EPS(–) *Lb. delbrüeckii* subsp. *bulgaricus* strain with EPS(+) *Lb. rhamnosus* JAAS8 strain during yoghurt production contributed to a 16–21% increase of viscosity and to increased water holding capacity in the fermented milk [90].

A weak correlation was demonstrated between yoghurt texture and EPS concentration [71]. The major factors which affect the texture of EPS-containing yoghurts are interactions of these compounds with casein which vary depending on the EPS structure and product acidity (pH) [7]. In turn, Ruas-Madiedo *et al.* [76] showed that the compactness and cohesiveness of yoghurt produced with EPS(+) strains decreases along with the increasing EPS concentration. The appearance of EPS before aggregation of casein micelles caused lower curd compactness.

To determine the effect of EPS addition on the microstructure and rheological properties of yoghurts, purified EPS were added to milk intended for yoghurt production (in concentrations of 0.01–0.03%. Viscosity, water holding capacity, hardness, and microstructure of yoghurts were strongly dependent on EPS concentration. The best water holding capacity (i.e. the least syneresis) was found in the yoghurt produced with 0.01% EPS. In turn, the 0.03% addition of EPS caused greater syneresis – likewise in EPS-free yoghurt. Also in terms of rheological properties, the best turned out to be the yoghurt with 0.01% addition of EPS [91]. In turn, yoghurt produced with yoghurt cultures and the EPS(+) *Lb. kefiranofaciens* ZW3 strain had a higher viscosity compared to the traditional yoghurt. No syneresis was observed during its storage (1 month) at room temperature, which indicates the advisability of using adjunct EPS(+) cultures in traditional yoghurt starters [3].

4.2. Effect of EPS on the quality of other fermented milk drinks

Kefir is a traditional, slightly sparkled fermented milk, popular in countries of Eastern Europe. It contains ca. 0.1–1.0% ethanol, depending on the fermentation activity of yeast. In the traditional method of kefir production, kefir grains containing homo- and heterofermentative LAB, yeast and acetic acid bacteria are added to milk. Cells of bacteria constituting kefir grains are built into the EPS matrix [16]. Today, however, kefir starters are used in kefir production instead of kefir grains which were filtered from the final product after completed fermentation. Although kefir starters contain yeast, they are incapable of fermenting lactose. For this reason, modern industrial kefirs are slightly saturated with CO_2 and contain trace amounts of alcohol. A recent trend assumes simplification of production technologies of fermented milk drinks, e.g., kefir or buttermilk, mainly owing to the concerns for their stability and economy. These practices may, however, lead to the deterioration of flavor and biodiversity of these products. A few of EPS(+) LAB strains, e.g., *Lb. plantarum* KF5 synthesizing an EPS composed of mannose, glucose and galactose [87], as well as *Lb. kefiranofaciens* ZW3, producing a heteropolysaccharide constituted by only glucose and galactose [85, 86], were isolated from kefir. The EPS isolated during fermentation of cow's milk with kefir grains consisted of glucose and galactose monomers (in the ratio of 1:0.43), whereas that isolated during soybean milk fermentation was composed of the same type of monomers but in the ratio of 1:1.14 [55]. In turn, EPS isolated from kefir produced based on soybean milk by Botelho *et al.* [9] contained only glucose monomers.

The EPS(+) cultures are also applied for the production of dahi – a traditional yoghurt made based on buf-

falo, cow or goat milk, popular throughout South Asian countries, such as, Bangladesh, India, Nepal, Pakistan, Sri Lanka, etc. [66]. The fat content of dahi is usually between 3.5–8%, but dahi assortment includes also its low-fat milk versions. Unfortunately, like for yoghurt and other dairy products, the low fat content of dahi has a negative effect on its quality, including lack of flavor, weak body and unstable texture [80]. The use of EPS(+) *Lc. lactis* subsp. *lactis* PM23, *S. thermophilus* ST and *Lc. lactis* NCDC 191 for the production of low-fat dahi was reported to improve its texture and flavor [8].

Selected strains of mesophilic LAB (*Lc. lactis* subsp. *cremoris, Lc. lactis* subsp. *lactis, Lc. lactis* subsp. *lactis* biovar diacetylactis*, Ln. mesenteroides* subsp. *cremoris*, and *Ln. mesenteroides* subsp. *dextranicum*) and thermophilic LAB (*S. thermophilus*, *Lb. delbrüeckii* subsp. *bulgaricus*, and *Lb. helveticus*), synthesizing slime EPS, and alternatively strains of yeast and molds are used in the Scandinavian countries to manufacture Nordic ropy milk type products, e.g., Långfil, tette, and viili. To manufacture these drinks, milk inoculated with the starter culture is fermented at relatively low temperature of 15–25°C even for several dozen hours. The low fermentation temperature needed to produce these drinks facilitates better EPS synthesis at the temperature significantly lower than the optimal growth temperature and the sub-optimal temperature as well [62, 72]. These types of fermented milks are also popular in Russia and Mongolia [16]. Viili is manufactured with the use of *Lc. lactis* subsp. *lactis* biovar diacetylactis*, Ln. mesenteroides* subsp. *cremoris* and mold *Geotrichum candidum*, which grows on the product's surface. The EPS isolated from viili contains rhamnose, glucose and galactose in the molar ratio of 1:1.45:1.75, and a phosphate group attached to a D-galactopyranosyl residue [16].

4.3. Effect of EPS on the quality of cheeses

Results of studies conducted so far have demonstrated the use of EPS(+) LAB strains to be a good alternative for the production of low-fat cheeses [12, 82]. The high water holding capacity of EPS has a positive effect on increasing viscosity and improving the texture and consistency of such cheeses [16, 25]. In many countries, some varieties of ripening rennet cheeses are produced in the low-fat version to meet demands of consumers who prefer low-caloric foods. A frequent defect of these cheeses is their little intense taste and rubbery, dry and grainy texture. A challenge faced by producers is to maintain their mouthfeel and texture similar to these of the full-fat cheeses, by modifying the production technology. Such attempts have been described in literature and involved the use of EPS(+) LAB [16, 20].

Traditional fresh Egyptian cheese (Karish) manufactured by acid coagulation of skim milk often shows texture defects typical of low-fat cheeses. In the microscope image, Karish cheese produced with the addition of EPS(+) cultures of LAB had a strongly porous structure and EPS were visible as clusters of fibers inside large pores. In turn, cheeses made using mutants of the same strains but incapable of producing EPS had a compact structure with small pores. The curd formed with the use of EPS(+) cultures was less rigid and more susceptible to deformations, compared to the curd formed using EPS(–) cultures [1, 31–33]. Fresh cheeses made with the use of EPS(+) cultures had smoother consistency than those produced without the addition of these cultures. Such improvement of textural properties may increase consumer acceptance of low-fat products. In addition, the use of EPS(+) cultures for the manufacture of fresh cheeses may increase consumer acceptance of cheeses containing fruits or vegetables owing to their improved spreadability and smoothness [1]. Hahn *et al.* [27] reported that the use of EPS(+) strains for the production of fresh cheeses may successfully reduce the formation of rough particles, thereby allowing to avoid the addition of hydrocolloids. Fresh cheeses are highly susceptible to syneresis owing to their high water content. The use of *S. thermophilus* synthesizing slime EPS in the manufacture of Mexican Panela cheese caused increased water retention in the cheese matrix. This enabled increasing production yield and reducing syneresis, as well as manufacturing cheeses with a smoother, creamy structure [38]. The use of EPS(+) *Lb. rhamnosus* CRL 1808 strain to manufacture spreadable acid-rennet goat cheeses improved their texture without the addition of gums or stabilizers [18].

Mozzarella cheese is manufactured with the use of thermophilic starter cultures including *S. thermophilus* and *Lb. helveticus* or *Lb. delbrüeckii* subsp. *bulgaricus*. It is the most common cheese topping on pizza, and therefore should satisfy highly specific functional requirements, including sufficient melting, adequate stretchability, and easy shredding, in particular. Unfortunately, low-fat Mozzarella cheeses do not develop good melting properties. Among the solutions to this quality problem is the use of EPS(+) starters. The low-fat Mozzarella cheeses manufactured with EPS(+) starters showed from 2 to 7% higher moisture, good consistency and better functional properties (melting) than these produced without EPS(+) cultures. It was also demonstrated that the use of strains synthesizing ropy EPS allowed obtaining higher water content in cheeses than the use of strains producing capsular EPS. The use of the first causes significant EPS diffusion in whey and an increase in whey viscosity, which may pose problems during its further processing (evaporation, drying) [68].

The application of LAB synthesizing EPS may be a potential means for increasing the water content and improving the textural properties of low-fat Cheddar

type cheeses. Considering that LAB have the GRAS status (i.e. are Generally Recognized As Safe for health on the basis of use for a long time), the use of an EPS(+) strain for the production of fermented foods is more propitious than the use of polysaccharides synthesized by other bacteria (e.g., dextran, gellan, pullulan, xanthan, alginates) [11]. Costa *et al.* [10] demonstrated that slime EPS synthesized by an adjunct culture of *Lc. lactis* subsp. *cremoris* DPC 6532 used in the manufacture of a semi-fat Cheddar cheese did not interfere with the milk coagulation process, but significantly affected syneresis reduction shortly after curd cutting. The EPS were uniformly distributed throughout the cheese matrix and specifically located near the aqueous pores, probably binding the moisture and causing the observed decrease in syneresis. This was reflected in the chemical composition of the cheeses with EPS, which were characterized by a higher water content but lower contents of protein and calcium, and by a higher yield.

Feta cheese manufactured using the EPS(+)cultures has an open microstructure with large pores which are partly or completely filled with a crosslinked structure, e.g., water with suspended EPS. In turn, Feta cheese produced with EPS(–) cultures has a compact structure. Aggregates of casein micelles in the cheese made with EPS-producing culture appeared to be more fused than these in the cheese made with the EPS-non-producing cultures [31, 32]. In earlier studies, Hassan *et al.* [30] also observed that the structure of Feta cheese produced with EPS(–) cultures had larger casein agglomerates than the cheese without EPS, and noticed a lower number of fat globules in the cheese made with EPS(+) cultures.

Investigations on the effect of EPS on the quality of cheeses were mainly conducted with products having a reduced or low fat content, while little is known about EPS effect on the quality of full-fat cheeses. Most likely, this effect was not investigated as the full-fat cheeses are soft and smooth enough, and free of defects typical of the reduced-fat cheeses.

5. Health-promoting properties of exopolysaccharides

Apart from the sensory benefits stemming from the EPS presence in dairy products, many EPS(+) LAB strains exhibit traits of probiotics [12, 16]. The probiotic activity of LAB strains is believed to be partly associated with the activity of biopolymers they produce [84]. The probiotic effect is then due not only to the activity of viable microorganisms, but also to the activity of their metabolites, including EPS (the so-called postbiotics). Beside the prebiotic effect of EPS [78], they are also claimed to have antibacterial activities [35, 41] and many health-promoting properties like: anti-carcinogenic, antioxidative, immunomodulatory, and reducing blood cholesterol [24, 26, 42, 43, 50, 51, 56, 59, 64, 65, 79, 84].

The mechanism of the anti-carcinogenic activity of EPS has not been fully elucidated, yet. According to one of the theories, EPS induce apoptosis of cancer cells by removing reactive oxygen species from their mitochondria [24, 51, 94]. The oxidative stress plays a key role in cancer pathogenesis. Levels of antioxidants and reactive oxygen species are correlated with the development and malignant transformation of cancer cells [37, 51]. Given that the EPS are capable of promoting antioxidative transformations and removing reactive oxygen species in cancer cells, they may as well inhibit their proliferation [51]. An EPS isolated from *Lb. helveticus* MB2-1 and built of three fractions: LHEPS-1, LHEPS-2 and LHEPS-3, inhibited the proliferation of human stomach cancer cells (cell line BGC-823). This activity was revealed for crude EPS (non-purified) and for all its three fractions. All of the three fractions were also capable of scavenging free radicals and chelating iron ions [50–52]. Liu *et al.* [56] demonstrated that EPS produced by *Lb. casei* 01, in the concentration from 0.005 to 0.050 g/l, exhibited a high antiproliferative against HT-29 human colon cancer cells, but simultaneously had no adverse effect on enteric cells. It is speculated that this activity involves both regulating enteric cells work and reducing cytotoxicity of procarcinogens. EPS of the probiotic *Lb. plantarum* NRRL B-4496 was active *in vitro* against tumor cell lines: Caco (intestinal carcinoma cell line), HeLa (cervical carcinoma cell line), HCT116 (colon carcinoma cell line), Hep-G2 (liver carcinoma cell line), MCF-7 (breast carcinoma cell line), and HEp2 (larynx carcinoma cell line) [29]. Results of the above-cited works allow hypothesizing that the anti-carcinogenic properties of EPS are ascribed to their activity as effectors inducing the immune response of the host body.

The slime EPS produced by *Lb. paraplantarum* BGCG11 was shown to exhibit anti-inflammatory and anti-suppressive properties [64]. This *in vitro* study demonstrated that during stimulation of peripheral blood mononuclear cells with purified EPS, the cytokine profile was similar to that induced by stimulation with viable cells of *Lb. paraplantarum* BGCG11.

Furthermore, EPS isolated from *Lc. lactis* subsp. *lactis*, and its derivative with selenium (Se-EPS) were capable of scavenging free superoxide anions (O_2^-) and hydroxyl radicals. In addition, they enhanced the activity of selected enzymes, e.g., catalase, superoxide dismutase and glutathione peroxidase, while reducing the level of malondialdehyde (MDA; an indicator of adverse lipid peroxidation) in blood serum and liver, and displaying immunomodulatory properties [26].

The hydrogen peroxide radical scavenging activity was also reported for EPS produced by *S. thermophilus* CC30 [40]. Investigations on the immunomodulatory properties of EPS produced by LAB (*Lc. lactis* subsp. *cremoris*, *Lb. delbrüeckii* subsp. *bulgaricus*, and *Ln. mesenteroides*) demonstrated some EPS to be capable of inducing cytokine synthesis [84] and modifying selected functions of macrophages and splenocytes [42]. EPS derived from yogurt obtained by fermentation using *Lb. delbrüeckii* subsp. *bulgaricus* OLL1073R-1 exerted immunostimulatory effects in mice [61]. Yogurt and semi-hard cheeses represent suitable food matrices for the delivery of the hypocholesterolemic EPS-producer strain *Lb. mucosae* DPC 6426 [77]. Works addressing kefiran indicate its antibacterial properties, ability to accelerate wound healing [73], and its potential to reduce blood pressure and cholesterol level in blood serum [59].

Apart from the longstanding health benefits resulting from the use of EPS(+) strains in the manufacture of dairy products, the EPS may also beneficially affect consumer physiology. Presumably, increased viscosity of EPS-containing fermented milk may prolong the time of its retention in the gastrointestinal tract, which is beneficial for, e.g., temporary gut colonization by probiotic bacteria. Another example of the putative health benefits of some EPS is their degradation in the colon to short-chain fatty acids (SCFAs) by enteric microflora. The SCFAs, and butyric acid in particular, provide energy to intestinal epithelium cells, and some of them prevent colon cancer [95].

6. Conclusions

In the food industry, the role of LAB capable of producing EPS may increase considering their effects on the rheological and textural properties of fermented food products. The EPS synthesized by LAB differ in their chemical composition and structure. Their production is relatively low (up to 0.1%), however they improve the consistency, stability and the widely understood quality of the final product. LAB capable of EPS production may find application in the manufacture of fermented milk products in countries in which the use of stabilizers is either limited or banned by law. The application of adjunct EPS(+) cultures in the production of fermented milks allows reducing the addition of milk powder and other thickening agents and offers vast possibilities for diversifying production, inscribing into the "clean label" trend, and meeting consumer demands for health-promoting and/or dedicated foods. EPS(+) cultures could also be applied in cheesemaking, especially in the case of low-fat fresh cheeses. Such a solution would prevent whey syneresis, but simultaneously give the sensation of some "fatness". Besides technological benefits, the use of EPS(+) LAB for the manufacture of fermented dairy products has a positive effect on human health, involving mainly elongation of fermented milk retention in the gastrointestinal tract and thereby promoting gut colonization by probiotic bacteria. The EPS are usually prebiotics, however they also exhibit anti-ulcer, immunomodulatory, anticarcinogenic activities and reduce blood cholesterol.

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